IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Lars Lilljequist et al.
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For	: Novel Crystalline Forms of 2,3-Dimethyl-8-(2,6-Dimethylbenzyl-amino)-N-Hydroxyethyl-Imidazo-[1,2-a] Pyridine-6-Carboxamide Mesylate Salt
Examiner	: Niloofar Rahmani
Group Art Unit	: 1625

I hereby certify that this paper is being transmitted via the Electronic Filing System to the U.S. Patent and Trademark Office on the date indicated below.

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Declaration of Ursula Hohlneicher
(Under 37 C.F.R. §1.132)

Sir:

I, Ursula Hohlneicher, declare as follows:

1. I am a citizen of Germany. I graduated in 1994 from the Universität zu Köln (University of Cologne, Germany) with a Ph.D. degree in Chemistry.

2. The Assignee of the referenced application is AstraZeneca AB. Since April 2008, my present position with AstraZeneca AB is Global Programme Leader in Pharmaceutical and Analytical Development. In my previous positions at AstraZeneca AB, I was a Manager of Solid State Analysis and a Manager of Preformulation. Both of these positions were in the Department of Preformulation and Biopharmaceutics, and I held these positions from 1999-2006. Prior to these positions, I held a number of positions in analytical chemistry at Astra Draco AB, a subsidiary of AstraZeneca AB. My Curriculum Vitae is attached to this Declaration as Exhibit A.

3. I have read and understood the referenced patent application and I am familiar with the invention described and claimed therein. Specifically, the claimed invention is directed to

the new and nonobvious mesylate salt of 2,3-dimethyl-8-(2,6-dimethylbenzylamino)-N-hydroxyethyl-imidazo-[1,2-a]pyridine-6-carboxamide and crystalline forms thereof. Unexpectedly, Applicants have discovered that the claimed mesylate salt has superior properties compared to the compound in the form of a free base or an HCl salt. In this regard, Applicants have conducted biological tests demonstrating that the mesylate salt has higher bioavailability and faster absorption than the free base or the HCl salt. By this Declaration, I hereby submit comparative data which supports the showing of unexpected results and advantages of Applicants' invention. The comparative data, as described below, is attached to this Declaration as Exhibit B.

4. The attached data in Exhibit B compares the *in vitro* dissolution behavior of 2,3-dimethyl-8-(2,6-dimethylbenzylamino)-N-hydroxyethyl-imidazo-[1,2-a]pyridine-6-carboxamide mesylate salt, as encompassed by the pending claims, to the corresponding free base and the hydrochloride salt. The study examined the dissolution rate of each compound in human intestinal fluid using the rotating disc method.

5. As can be seen in Exhibit B, the mesylate salt according to the pending claims exhibits an superior dissolution behavior as compared to the corresponding free base or HCl salt. The results show that the dissolution rate of the mesylate salt in human intestinal fluid was consistently several folds higher as compared to the free base or HCl salt. For example, in Figure 1, the comparative data demonstrates that the mesylate salt attains a concentration of 50 μg/ml in intestinal fluids after approximately 20 seconds, whereas the free base and HCl salt do not reach this concentration even after 900 seconds (15 minutes). This superior dissolution rate indicates that the mesylate salt will have a higher bioavailability and faster absorption *in vivo*.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

16 July 2008
Date

Ursula Hohlneicher

Exhibit A- Curriculum Vitae of Ursula Hohlneicher

Family Name	Hohlneicher
Given Name	URSULA Renata Maria
Date of Birth	November 24, 1964
Citizenship	German
Education and /or academic degree	PhD, Universität zu Köln (Cologne, Germany) 1994
Present Position	Global Programme Leader for Leopard (a global AstraZeneca IS Implementation Programme)
Previous Positions	Regional Team Manager Information & Systems Management Business Support, PAR&D Sweden 2006 - 2008 Manager Solid State Analysis in Preformulation&Biopharmaceutics, PAR&D, AstraZeneca R&D Mölndal 2001 - 2006 Manager Preformulation in Preformulation&Biopharmaceutics, PAR&D, AstraZeneca R&D Lund, Sweden 1999 - 2001 Assistant Director, Dept. of Analysis- Substance, Astra Draco AB, Lund, Sweden 1997 - 1999 Assistant Director, Analytical Chemistry, Astra Draco AB, Lund, Sweden 1996 - 1997 Research Scientist, Analytical Chemistry, Astra Draco AB, Lund, Sweden 1995 - 1996 Senior Scientific Assistant, Institut für Organische Chemie II, Universität zu Köln, Germany 1990 - 1995

Publications:

K. Taraz, R. Tappe, H. Schröder, U. Hohlneicher, I. Gwose, H. Budzikiewicz, G. Mohn, J.-F. Lefevre
Z. Naturforsch. **46c**, 527 (1991).
Ferribactins - the biogenetic precursors of pyoverdins

U. Hohlneicher, R. Hartmann, K. Taraz, H. Budzikiewicz
Z. Naturforsch. **47b,** 1633 (1992)
The structure of ferribactin from *Pseudomonas fluorescens ATCC 13525*

U. Hohlneicher, R. Hartmann, K. Taraz, H. Budzikiewicz
Z. Naturforsch. **50c**, 337 (1995).
Pyoverdin, ferribactin, azotobactin - a new triade of siderophores from *Pseudomonas chlororaphis ATCC 9446* and its relation to *Pseudomonas fluorescens ATCC 13525*

U. Hohlneicher, M. Schäfer, R. Fuchs, H. Budzikiewicz
Z. Naturforsch. **56c**, 308 (2001).
Ferribactins as the Biosynthetic Precursors of the *Pseudomonas* Siderophores Pyoverdins

Ursula Hohlneicher
Mölndal, Sweden

Exhibit B- Comparative Data

Dissolution experiments and a dog study were performed in order to evaluate the performance of different forms of the active compound. *In vitro* dissolution experiments were performed and two immediate release formulations, one containing the mesylate salt and the other containing the HCl salt, were tested *in vivo* in fasted pH modified dogs.

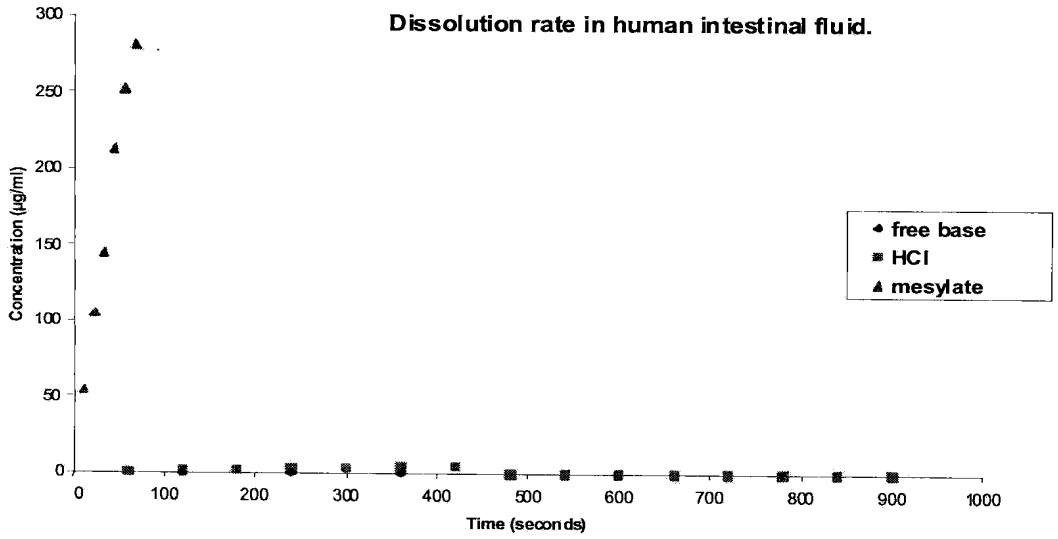
For the *in vitro* studies, all tablets contained either the HCl salt or mesylate salt of the active compound with the dose being 75 mg. The following immediate release ("IR") formulations were used in the study:

TR12205: IR formulation containing the HCl salt; and
TR11281: IR formulation containing the mesylate salt

1. In vitro results

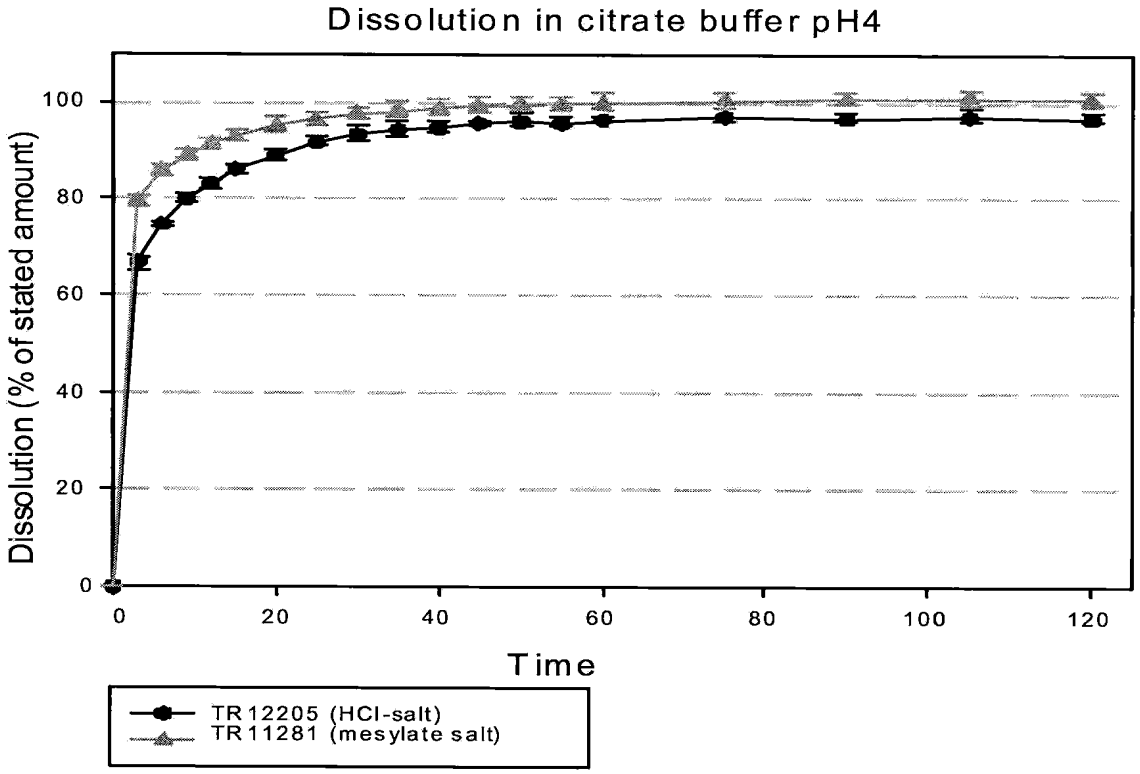
The dissolution rates of the two salts were evaluated using the rotating disc method. The results show that the dissolution rate in human intestinal fluid was several folds higher for the mesylate salt compared to the HCl salt or the free form (see Figure 1). This difference indicates that the mesylate salt will have a higher bioavailability and faster absorption *in vivo*.

Figure 1- Dissolution rate of compound in human intestinal fluid using the rotating disc method.



In contrast, the two tablet formulations TR12205 (HCl salt) and TR11281 (mesylate salt) had similar *in vitro* dissolution profiles in citrate buffer at pH 4. The dissolution rate was slightly slower for the formulation containing the HCl salt formulation in the citrate buffer method, as shown in Fig. 2.

Figure 2- Average dissolution profiles of TR12205 (HCl salt) and TR11281 (mesylate salt) in citric buffer pH 4.

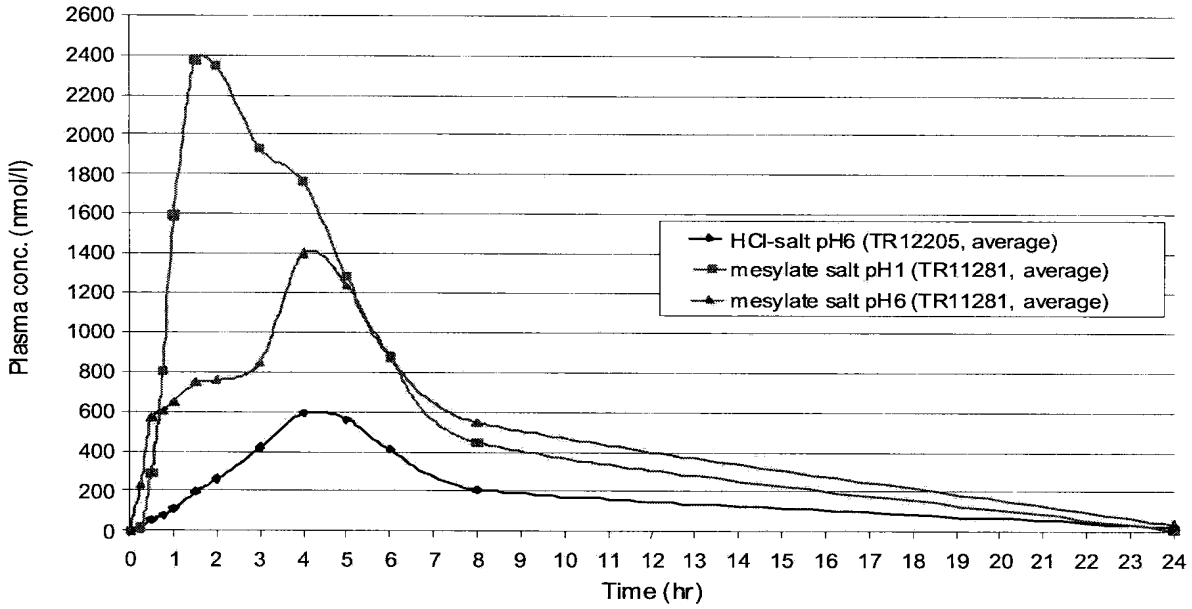


***2. In vivo* results**

Tablets containing the mesylate salt were administered p.o to fasted dogs with reduced gastric pH (pH 1) and elevated gastric pH (pH 6) prior to tablet dosing. Tablets containing the HCl salt were administered to dogs having elevated gastric pH (pH 6).

Significant differences were detected for both the response variables AUC and Cmax. The mesylate salt formulation both at pH 1 and at pH 6 was better than the HCl salt formulation at pH 6 (see Figure 3).

Figure 3- Average plasma profiles after p.o administration of IR formulations containing 75 mg of active compound to fasted pH modified dogs.



The *in vitro* dissolution data from the rotating disc method discriminated well for the differences shown in exposure *in vivo*. The dissolution data in citrate buffer pH 4 are less suitable to indicate how the salts perform *in vivo*.

Experimental Data

I. In Vitro Dissolution Studies in Citrate Buffer

Three formulations of 2,3-dimethyl-8-(2,6-dimethylbenzylamino)-N-hydroxyethyl-imidazo-[1,2-a]pyridine-6-carboxamide ["the active compound"] as mesylate or HCl salt were tested. Regarding compositions of tablets of mesylate salt and HCl salt, respectively, see Table 1.

A. Principle

Individual tablets are tested for dissolution in (a) human intestinal fluid; and (b) citric acid buffer at pH 4.0 and 37°C, using USP apparatus 2 at 75 rpm. The amount of each compound dissolved is monitored during 120 minutes using a spectrophotometer equipped with fiber optic probes at 300 nm. A fiber optic probe is immersed in each dissolution vessel during the analysis.

B. Equipment

Spectrophotometer with at least 6 fiber optic dipping probes of a well-defined path length.

C. Reagents and solutions for testing

Water used for preparation of the reagents is filtered through a water purification unit. All chemicals are of analytical grade unless otherwise stated. Suitable weighing and dilutions are exemplified below.

1. Citric acid 1 M
Dissolve 210 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in 1000 mL water.

2. tri-Sodium citrate 1 M
Dissolve 294 g of tri-sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in 1000 mL water.

3. Sodium citrate buffer pH 4.0 (I=0.15) = Dissolution medium
Mix 1500 mL citric acid 1 M (1) and 1000 mL tri-sodium citrate 1 M (2) and dilute to 25 L with water.

4. Methanol

5. Active compound in form of free base, HCl salt, and mesylate salt.

6. Active compound standard solution
Dissolve the active compound (5) in methanol (4), no more than 2% of the total volume, and dilute with the dissolution medium (3) to a final concentration corresponding to the nominal strength of the tablet. The minimum volume of standard solution should be 250 mL.

D. Procedure

Immerse the fiber optic probes into the standard solution (6) and measure the absorbance in a scan ranging from 300 nm to 400 nm.

Transfer 900 mL sodium citrate buffer pH 4.0 (I=0.1) (3) or human intestinal fluid (7) to each of the 6 dissolution vessels and equilibrate at 37°C. Assemble the apparatus and set the paddle speed to 75 rpm. In order to avoid bubble formation on the probes, deaerate the dissolution media for 10 min directly in the vessels (e.g., with He-gas) or dispense deaerated media. Immerse the fiber optic probes into the dissolution vessels at a height, midway

between the surface of the dissolution medium and the top of the rotating paddle blade, and 3 cm from the paddle shaft centre. The probes should be oriented to maximize the flow through the sampling cells. Transfer 1 tablet to each dissolution vessel at suitable time intervals. Measure the absorbance in a scan ranging from 300 nm to 400 nm using a suitable path length. A suitable path length for 12.5-75 mg tablets is 4 mm.

E. Calculations

$$\frac{A \cdot c_s \cdot V \cdot 100}{A_s \cdot SA}$$ = active compound dissolved (as % of stated amount)

A = net absorbance (A_{300nm}-A_{400nm}) of sample solution (AU)
A_s = absorbance (A_{300nm}) of active compound of the standard solution (6) (AU)
SA = stated amount of sample (mg), calculated as

$$\frac{SA_{AR-H044277\,XX} \cdot M_{AR-H044277\,AW}}{M_{AR-H044277\,XX}}$$

$M_{AR-H044277\,XX}$ = molecular weight of free base = 366.5 g/mol
$M_{AR-H044277\,AW}$ = molecular weight of mesylate salt = 462.6 g/mol
V = volume of the dissolution medium (mL)
c_s = concentration of active compound standard solution (mg/mL)

II. Dissolution studies in human intestinal fluid (HIF)

The human intestinal fluid was provided by Uppsala University. It was collected by the Loc-I-Gut technique from volunteers (age 18 - 40) from the proximal jejunum after 10 h of fasting.

For more details on the technique see:

1. H. Lennernas, Ö. Ahrenstedt, R. Hällgren, L. Knutsson, M. Ryde, and L. Paalzow. Regional jejunal perfusion, a new in vivo approach to study oral drug absorption in man. Pharm res. 9:1243-1251 (1992).

2. L. Knutson, B. Odlind, and R. Hallgren. A new technique for segmental jejunal perfusion in man. Am J Gastroenterol. 84: 1278-84 (1989).

The human intestinal fluid used has a pH between 6.5 and 7. Volumes used for dissolution testing are 5 ml.

III. In Vivo Animal Studies

A. Formulations

Two IR formulations of 2,3-dimethyl-8-(2,6-dimethylbenzylamino)-N-hydroxyethyl-imidazo-[1,2-a]pyridine-6-carboxamide ["the active compound"] as mesylate or HCl salt without any base element were tested in dogs.

Table 1 Compositions of TR11281 (Mesylate) and TR12205 (HCl)

Ingredient (%)	TR11281	TR12205
Mesylate salt	31.8	0
HCl salt	0	27.7
MCC	36.3	40.4
Mannitol	1.6	1.6
L-HPC LH-22	6.2	6.2
HPC-LF	2.1	2.1
MCC coarse	21	20
PRUV®	0	2.0
Na stearyl fumarate	1.0	0

MCC = microcrystalline cellulose
HPC = hydroxypropyl cellulose

B. Administration routes and doses
75 mg tablets were administered p.o

C. Animal model

Animal species	Dog
Breed	Labrador
Sex	Male
Number	4
Age/Weight	3–8 years, 30 – 35 kg
Surgery	VF cannula
Fast/fed	Fasted 22 hour prior to study and fed 4 hours after dosing.
Water	ad lib at all times.

D. Study design
In this study 4 male dogs equipped with a cannula in the stomach (VF cannula) were used. The dogs were fasted over night but water was allowed at all times.

Before the start of the experiment each dog was weighed and a blood sample was collected (t=0 min). In all experiments the VF cannula was opened and the stomach was emptied of its contents. The pH electrode was inserted together with an administration of either 55 ml of HCl/KCl solution (pH 1 study) or with 75 ml of water (pH 6 study). The pH was modified and measured for 2 hours after start of the experiment (see chapter 3.7). The tablets were administered along with 20 ml HCl/KCl (pH 1 study) or together with 75 ml of water (pH 6 study), one tablet each experimental day. Blood samples of 2 ml were taken at specific time points. Four experiments in a 2-week period were conducted and then the dogs were allowed to rest for at least 3 weeks before the next experiment. A maximum of 1 % of the total blood volume in the dogs were withdrawn during each experiment.

The gastric pH was modified to around pH 1 with the aid of p.o administration of 55 ml HCl/KCl solution. pH 1 was reached within 5 minutes.

The gastric pH was modified to around pH 6 with the aid of an i.v administration of omeprazole (Losec® 40 mg, 1 mg/kg). Losec® was administered directly into a foreleg vein during a period of 2 minutes. pH 6 was expected to be attained within 30-60 minutes after i.v administration. However, if the pH did not reach above 4 within 90 minutes the experiment was terminated.

E. pH measurement
The regular stopper used to close the VF cannula was replaced by a stopper with a drilled hole where a pH electrode was inserted and approximately 5 cm of the electrode reached into the stomach. The electrode was held in place with a specially manufactured screw cap and rubber tubes prevented leakage through the drilled hole in the stopper. The pH-electrode was connected to a pH-meter (Digitrapper pH, Medtronic A/S) that continuously measured the gastric pH.

The recording started after p.o administration of the IR formulation and continued for 120 minutes.

F. P.o administration

1. TR11281- Mesylate Salt
When the pH recorder showed the desired pH level of approximately 1, the IR formulation TR11281 was administered p.o followed by another 20 ml of HCl-KCl administered p.o with the use of an orogastric tube.

2. TR12205- HCl Salt
When the pH recorder showed the desired pH level (see chapter 3.7), 65 ml of water was given p.o with the use of an orogastric tube and the IR formulations were administered followed by another 10 ml of water. Administration of the IR formulations were set to occur earliest 30 minutes after Losec® infusion.

G. Blood sampling
Blood samples were collected from veins in front leg, back leg or neck. Prior to administration a first blood sample was taken (t=0). 2 ml blood samples were then collected at: 15, 30, 45 minutes and 1, 1.5, 2, 3, 4, 5, 6, 8 and 24 hours after administration of the active compound.

A total of 13 samples at 2 ml each are collected from each dog and this gives the total amount of 26 ml per dog each experimental day. For the entire study there were 52 samples taken per dog and this gives a total number of 208 samples.

H. Sample treatment and analysis
Approximately 2 ml of blood were collected in heparinized Venoject sampling tubes. Turning the sampling tubes a few times gently mixed blood and heparin. Within 30 minutes after blood sampling the sampling tubes were centrifuged at 3000g for 10 minutes at 4°C. The plasma was transferred to 4 ml Cryovial™ tubes and frozen in a -20°C freezer. The concentrations of the active compound in plasma were determined by HPLC with fluorescence-detection

I. Calculations
Standard techniques were used to assess the various bioavailability parameters with the assistance of the computer program WinNonLin. The maximum concentration of the active compound in plasma, Cmax, the time to reach Cmax, Tmax, were identified for each subject and formulation. The area under the plasma concentration time curve, AUC, (the area from time zero up to the last measurable plasma concentration calculated according to the linear trapezoidal method.

The absolute bioavailability, F (%), (IR formulations after p.o administration in relation to i.v administration of the solution (from study D9770-800)) was determined according to equation 1. F was calculated with each individual dog as its own reference.

F (%) = AUCp.o/AUCi.v x Dosei.v/ Dosep.o x 100 [equation 1]

The relative bioavailability, Frel (%), (IR formulations after p.o administration in relation to the reference tablet TR11281, pH 6 from study D9770-1100, was determined according to equation 2.

Frel (%) = AUCp.o/AUCp.o ref. x Dosep.o ref./ Dosep.o x 100 [equation 2]

Frel was calculated with each individual dog as its own reference, however the average value of the of the 2 measurements of the reference formulation were used.